

**CYP3A4\*1G gene Polymorphism on Javanese People**Em Sutrisna<sup>1,2\*</sup>, Iwan Dwiprahasto<sup>3</sup>, Indwiani Astuti<sup>3</sup>, and Erna Kristin<sup>3</sup><sup>1</sup>Doctoral Program of Medical and Health Science, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia,<sup>2</sup>Faculty of Pharmacy, Muhammadiyah University of Surakarta, Surakarta, Indonesia<sup>3</sup>Department of Pharmacology and Toxicology, Faculty of Medicine Universitas Gadjah Mada, Yogyakarta, Indonesia**Abstract**

Most of drugs are metabolized by cytochrome P 450 (CYP) enzyme. Cytochrome P450 3A4 is the cytochrome that is involved in metabolizing more than 60% of all medicine used in human. The variation of this CYP3A4 gene will affect the catalytic activity of this enzyme. Recently, CYP3A4\*1G in intron 10 was found in Chinese and Japanese population. There is a substitution of G to A at position 82266 in intron 10. The purpose of this research was to investigate the frequency of allele and genotype CYP3A4\*1G. Samples were taken from bloods of the subjects of the research. The examination of CYP3A4\*1G was conducted by RT-PCR method. As the results of this research, the frequency of CYP3A4\*1G in Javanese people is CYP3A4\*/1 0.25, CYP3A4\*1/1G 0.55 and CYP3A4\*1G/1G 0.20. Frequency of allele G: 0.53, allele A: 0.47. The Fisher's exact- test shows that the allele and genotype frequency is p. 1.000. The allele and genotype frequency of Javanese people is still in Hardy-Weinberg equilibrium.

**Keywords :** CYP3A4\*1G gene, polymorphism, Javanese people**Introduction**

The response to drugs delivery is influenced by many factors. These factors included drug's factors and host factors. Host factors that affect clinical response are age, nutritional status, and physiologic organs including genetics factors. Genetic variation that currently focused on attention is genetic polymorphism in individual that leads to differences in treatment response. Polymorphism in individual can influence the pharmacokinetic profile of drugs, including absorption, distribution, metabolism and elimination. This happens because these polymorphism can cause level of drugs does not achieve the expected therapeutic

levels (sub therapeutics), or even cause toxic therapeutics. Sub therapeutic levels can cause the treatment failure and toxic therapeutics level can lead to excessive drugs effect (Alvirevi *et al*, 2006)

CYP3A4 enzyme is present in liver and gastrointestinal tract (Tomaszewski *et al.*, 2008; Hsieh, *et al.*, 2001) and in renal and prostatic tissue (Tomaszewski *et al.*, 2008). Human liver contains the most abundant isoenzyme of CYP450 (Gao *et al.*, 2008). It has 18 isoform numbers with a molecular weight of 57,1kD (Tomaszewski *et al.*, 2008). CYP3A4 is involved in the metabolism of more than 50% of drugs in human (Van Schaik *et al.*, 2000 ; Wang *et al.*, 2005), among of these are alprazolam, amiodaron, amlodipin, amitriptilin, atorvastatin, deksametason, dextrometorpan, diazepam, digoksin, diltiazem, ketokonazole, ondansentron, terfenadin, progesterone, nateglinid and others (Tomaszewski *et al.*, 2008).

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Polymorphism can be found on the enzyme of drugs metabolism gene, drugs transport gene, and drugs target gene. Polymorphism that is most widely mapping is on drug metabolism gene. Most of drugs are metabolized by cytochrome P450 (CYP). From the various CYP that has been mapped, CYP3A4 metabolizes more than 60% of medicines given to human. The research on the Malaysia's population showed that there were 2,1% CYP3A4\*18 (Ruzilawati *et al.*, 2007). Mapping of the CYP3A4\*1G polymorphism has not been done in Indonesia and Malaysia.

The purpose of this research is to investigate the allele and genotype frequency of CYP3A4\*1G on Javanese people.

## Materials and Methods

### Subjects

The Javanese people patients that took cure in Balai Kesehatan Paru Masyarakat (BKPM) Klaten and Balai Kesehatan Paru Masyarakat (BKPM) Yogyakarta. Inclusion criteria were age >14 years, Javanese ethnic (three generation and over) while exclusion criteria was family relationships (father, mother, grandmother, grandfather, children and grandchildren).

### DNA isolation

DNA isolation was performed by using Wizard® genomic DNA purification kit Promega, which steps were done as referred to manufacture recommendation. Briefly, 100 µL of cell lysis solution was added in 300 µL of buffy coat samples were incubated 10 min. On room temperature, mix solutions were centrifuged 13.000 for 1 min. After supernatant was discarded, 300 µL of solution was added on the residue. The mixture was vortexed 10-15 sec. The result of the mixture was added by 1,5 mL of RNA solution, then was vortexed 10-15 sec. These mixtures were incubated on 37°C then stored in room temperature and added by 100 µL of protein precipitation solution. Mixture then was vortexed on 10-15 sec and

centrifuged 13.000 for 3 min on 37°C. Three hundreds µL of supernatant was taken and put into 1,5 mL tube then added by 300 µL isopropanol. The solution was mixed by inversion until the white treads-like strands of DNA form a visible mass, then centrifuged on 13.000 for 1 min at 37°C. The supernatant was discarded and the residue was added by 300 µL of 70% ethanol. It was mixed a few time and centrifuged on 13.000 for 1 min on 37°C. The ethanol was removed after inversion. The residue was added by DNA rehydration solution (100 µL for 300 µL sample volume) and incubated at 65°C for 60 min. Periodically, the solution is mixed by gently tapping the tube. The samples were stored at 4°C.

### Polymerase chain reaction CYP3A4\*1G

A total of 12,5mL master mix, 6,5 mL dH<sub>2</sub>O, forward 5'-CAC CCT GAT GTC CAG CAG AAA CT-3' and 2µL reverse 5'-AAT AGA AAG CAG ATG AAC CAG AGCC-3' and 2µL DNA (25µL total) were mixed and run by PCR. Amplification conditions as follows: 94°C for 7 min, the 35 cycles by 30 secs on 94°C, 1 min on 62°C, dan 1 min on 72°C with 5 min final extension at 72°C. After PCR amplification, 8 µL of PCR products of 287 bp was digested by 10 U *RsaI* for 12 h at 37°C. The digested PCR products were analyzed by electrophoresis on 2% agarose gel and detected by ethidium bromide. The bands of DNA fragments were visualized by UV light (Gao *et al.*, 2008)

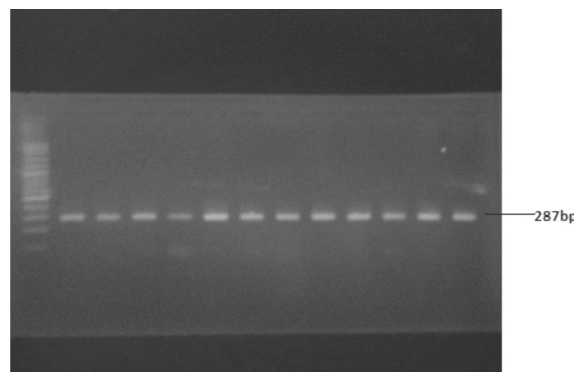


Figure 1. Electrophoregram of amplification products of CYP3A4

## Results

A total 60 subjects whom fulfilled the inclusion and exclusion criteria were used as test subjects.

The sample material was derived from blood buffy coat. The DNA purity was measured from ratio of absorbance at A260/280. The results of these samples were 1,7-2. DNA concentration was 50µg/mL with dilution. DNA which had isolated

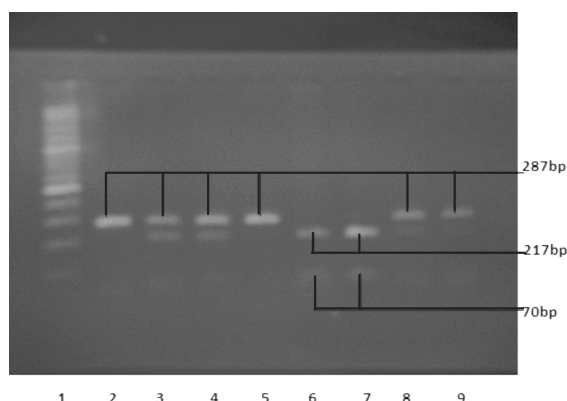


Figure 2. Detection of polymorphism in CYP3A4 \*1G using PCR-RFLP. Lane 1 is marker 100bp, lane 2. Uncut; lane 3,4,8,9 are from samples of CYP3A4 \*1/\*1G (heterozygotes); lane 5 is CYP3A4 \*1G/\*1G (mutant/variant type homozygotes) and lane 6-7 are samples of CYP3A4 \*1/\*1 (Wild type)

then amplified by RFLP-PCR. Results can be seen in Figure 1.

The digested RFLP-PCR-products that analyzed by 2% agarose gel electrophoresis is shown on Figure 2.

Figure 2 shows that lanes 3,4,8, and 9 contained digested product of RFLP-PCR with size 287 bp, 217 bp and 70 bp. These are CYP3A4\*1/\*1G (heterozygote variant type). Lane 5 (287bp) was not digested. This is homozygote variant/mutant type (CYP3A4\*1G/\*1G). The lane 6 and 7 shows digested product of 217 bp and 70 bp (wild type CYP3A4\*1/\*1).

The genotype and allele frequency can be seen in Table 1 and 2.

Table 2 showed that the sum of frequency of (G)+frequency (A)=1, and  $nG^2 + 2nGnA + nA^2=1$ . The frequency of genotype and allele was not deviated from

Table 1. Characteristic of subject test based on sex

gene	Sex		total
	Male	Female	
CYP3A4*1/1	6	8	14
CYP3A4*1/1G	20	14	34
CYP3A4*1G/1G	6	6	12
total	32	28	60

Table 2. Frequency of genotype& allele on subject test

Subjects	Frequency of genotype	Frequency of allele	P value
CYP3A4*1G			
- CYP3A4*1/*1	14(23.3%)		
- CYP3A4*1/*1G	34 (56.7%)		
- CYP3A4*1G/*1G	12 (20%)		
- Allel G (*1)		0.52	
- Allel A (*1G)		0.48	
			1.000*

\*test was done by Fisher's exact- test

Table 3. Frequency genotype and allele of CYP3A4\*1G from the others populations:

Populations	frequency allele			frequency genotype		Ref
	G (*1)	A(*1G)	*1/*1	*1/*1G	*1G/*1G	
Javanese	0.53	0.47	0.25	0.55	0.20	This study
China	0.724	0.276	0.51	0.43	0.06	Gao <i>et al.</i> , 2008
China	0.75	0.25	0.58	0.34	0.08	Dong <i>et al.</i> , 2011

Hardy-Weinberg equilibrium on p. 1,000. The comparison of this study with research in Chinese population can be seen in Table 3.

## Discussion

The result of this study showed that the frequency of polymorphism of CYP3A4\*1/\*1 was 0,25, CYP3A4\*1/\*1G was 0,55 and CYP3A4\*1G/\*1G was 0,20. Frequency of allele G was 0,53, while the frequency of allele A was 0,47. By comparing to two studies on Chinese populations, it showed that the frequency of variant type/mutant type CYP3A4\*1G/\*1G of Javanese population is much higher than Chinese population. The result of the genetics findings is different from anthropological studies. Anthropological studies states that the island of Java had inhabited by *Homo sapiens* since 40,000 years ago. The earliest inhabitants were austromelanesid ethnic that since 10.000 years ago were mongolized. This process became more intensive during last 1000 years. The Javanese were formed since 2168 years ago (Glinka, 2006), so there is a relationship between Mongolian and Javanese ethnics. The frequency of mutant/variant type in this research is relatively large (55% in heterozygotes and 20 % homozygous of CYP3A4\*1G/\*1G).

CYP3A4\*1G was determined by the substitution of 82266 G>A. Location of this variant is in intron 10 of the CYP3A4 gene. It was resulted in the change of amino acid from isoleucine into valine (I369V). Both of these amino acids have different polarity levels and molecular weight. The molecular weight of isoleucine (C<sub>6</sub>H<sub>13</sub>NO<sub>2</sub>) is 131.1736g/mol, while molecular weight of valine (C<sub>5</sub>H<sub>11</sub>NO<sub>2</sub>) is 117.1469g/mol. The amino acid with different unit's nature will reduce the catalyzing ability of cytochrome enzyme (CYP3A4) (Gao *et al.*, 2008). Thus, the effects of CYP3A4\*1G polymorphism is vary. After atorvastatin given, the mean percent reduction of total cholesterol plasma in individual with CYP3A4\*1/\*1 is 16,83±3%, 17,8±3,8 % (\*1/\*1G) and 20,9±5,0% (\*1G/\*1G). Conversely, a decreasing in

total cholesterol on simvastatin therapy showed no difference among these three variants (Gao *et al.*, 2008). Yuan *et al.* (2011) states that individual with variation of CYP3A4\*1G/\*1G has a lower metabolic rate of fentanyl than type CYP3A4\*1/\*1G and CYP3A4\*1/\*1. The fentanyl plasma levels of individual with CYP3A4\*1/\*1 (12,8 ±6,5 ng/mL) was significantly lower than CYP3A4\*1/\*1G (16,8± 9,0 ng/mL, p<0,01) and lower than individual with variant type CYP3A4\*1G/\*1G ( 28,1±9,5 ng/mL, p<0,01) (Yuan *et al.*, 2011). Variation effect of this polymorphism could occurred because of the substitution of guanine by adenine that occurred at 82266 (G82266A) in variant type CYP3A4\*1G/\*1G, which causes a change from isoleucine to valine (I 369 V) (Gao *et al.*, 2008). This amino acid change would reduce the ability of catalyzing cytochrome (CYP3A4) which may causes plasma drugs levels becoming higher. Therapy of drugs which are substrates of CYP3A4 together with other drugs, which both are inductor and inhibitors of CYP3A4, should also be tested to predict its effects.

Based on this result, therapy of drugs which are substrates of CYP3A4 must be carried carefully. Several drugs had been known as inhibitors and inductors. The drugs known as inductors CYP3A4 such as antiepileptic drugs (classphenobarbital, phenytoin, carbamazepine, felbamate, lamotrigine, oxcarbazepine, primidone, rufinamid & topiramate), cyclophosphamid, dexamethason, erythromisin, griseofulvin, lansoprazole, nevirapin, omeprazol, pioglitazone, prednisone, rifampin, troglitason. The drugs known as inhibitor of CYP3A4 are chloramphenicol, cimetidin, ciprofloxacin, flukonazole, itraconazole, nevirapin, norfloxacin, varikonazole, mibefradile, estradioland (Tomaszewski *et al.*, 2008). Therapy of drugs which are substrates of CYP3A4 together with its inhibitor may increase drugs plasma level. This can cause adverse effect. Conversely, the therapy of drugs that are substrates of CYP3A4 together with its



inductor may decrease drugs plasma level and can cause failure of the treatment.

As the conclusion, the frequency of CYP3A4\*1G in Javanese people were 0,25 for CYP3A4\*1/\*1, 0,55 for CYP3A4\*1/\*1G, and 0,20 for CYP3A4\*1G/\*1G. Frequency of allele G was 0,53 while allele A was 0,47. Allele and genotype frequency of Javanese people were still in Hardy-Weinberg equilibrium.

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